Palifosfamide: A Novel Molecule for the Treatment of Soft Tissue Sarcoma (STS)

An ESUN Article

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Abstract

Palifosfamide (Zymafos™ or ZIO-201) references a novel composition (tris formulation) that is the functional active metabolite of ifosfamide (IFOS), a bi-functional DNA alkylator being investigated as a potential therapy for the treatment of soft tissue sarcoma (STS). Palifosfamide is formulated by combining the tris (hydroxymethyl) amino methane (tris) salt of palifosfamide and a number of excipients to create the final drug product. Preclinical development of palifosfamide has included in vitro and in vivo studies demonstrating activity against various sarcomas, breast cancers, other solid tumors and leukemias, including several that are resistant to IFOS. Several clinical studies have been initiated in a variety of cancer types. A Phase I study in advanced cancers, using the original lysine formulation, has been completed. A two-stage Phase I/II Study in advanced sarcomas, introducing the tris salt formulation, has completed enrollment and data retrieval is ongoing. A Phase I study in combination with doxorubicin evaluating patients with advanced, refractory solid tumors for whom treatment with doxorubicin is considered medically acceptable, has completed enrollment and data retrieval is ongoing. Based on the result of the Phase I combination study, an international randomized Phase II study comparing palifosfamide in combination with doxorubicin versus doxorubicin alone in 1st and 2nd line patients with advanced STS has been initiated and is currently enrolling patients.

Introduction: What is Soft Tissue Sarcoma (STS) and what are the currently available treatments?

Soft-tissue sarcomas (STS) represent a rare and diverse group of tumors that are not very well understood. Although soft-tissue sarcomas account for <1% of all cancers, they represent a high percentage of cancer-related deaths worldwide (Ref. 3, Ref. 4, Ref. 5). STS tumors can occur anywhere within the body, originating in various soft tissues including fat, smooth or striated muscle, nerve/nerve sheath, vascular tissue, and other connective tissues; the extremities are the most common site of origin, accounting for approximately 50% of cases.

More than 50 histologic subtypes of soft-tissue sarcoma have been identified with similar clinical behavior (Ref. 6), with up to 100 fold variability in incidence rates (Table 1). The most common
types of sarcoma are malignant fibrous histiocytoma (MFH, now termed high grade undifferentiated pleomorphic sarcoma [HGUPS]), liposarcoma, and leiomyosarcoma. Also, the frequency of these subtypes differs depending on the site of disease. For example, leiomyosarcomas are the most common abdominal sarcoma, while liposarcomas and malignant fibrous histiocytomas are the most common type found in the legs. Determination of the histologic subtype is particularly important in terms of prognosis, as it aids in elucidating distinctive patterns of tumor behavior (Ref. 7, Ref. 8, Ref. 9).

### Table 1: Common Histologic Subtypes of Soft-tissue Sarcoma

<table>
<thead>
<tr>
<th>Histologic classification</th>
<th>Tissue of origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malignant fibrous histiocytoma</td>
<td>Upper and lower extremities; retroperitoneum</td>
</tr>
<tr>
<td>Liposarcoma</td>
<td>Fatty tissue (e.g., thigh, retroperitoneum)</td>
</tr>
<tr>
<td>Leiomyosarcoma</td>
<td>Smooth muscle tissue (e.g., intra-abdominal, uterus, retroperitoneum)</td>
</tr>
<tr>
<td>Fibrosarcoma</td>
<td>Fibrous tissue, typically in the thigh and trunk</td>
</tr>
<tr>
<td>Synovial sarcoma*</td>
<td>Synovial tissue lines the cavities of joints (tissues that connect muscle to bone), and bursae (fluid-filled, cushioning sacs in the spaces between tendons, ligaments, and bones) (e.g., knees, hands, and feet)*</td>
</tr>
<tr>
<td>Malignant peripheral nerve sheath tumors (e.g., neurofibrosarcoma)</td>
<td>Peripheral nerve sheath (anywhere in the body, but particularly in the lower extremities and retroperitoneum)</td>
</tr>
<tr>
<td>Gastrointestinal stromal tumor (GIST)</td>
<td>Gastrointestinal tract</td>
</tr>
</tbody>
</table>

Source: National Cancer Institute

*These and other STS cancer cells are actually often derived from pluripotential mesenchymal stem cells

Data from the US National Cancer Institute (NCI) show that from 2001–2005 the median age at diagnosis for cancer of the soft tissues (including the heart) was 57 years. Approximately 10.3% were diagnosed under age 20; 10.0% were between ages 20 and 34; 11.3% between 35 and 44; 14.9% between 45 and 54; 15.5% between 55 and 64; 15.5% between 65 and 74; 16.3% between 75 and 84; and 6.2% were 85 years or older (Ref. 7). Early stage STS are generally curable. Conversely, a late diagnosis, which is usually accompanied by extensive metastatic disease, may be difficult to cure. Approximately 40% to 50% of STS patients develop distant metastases. Patients diagnosed with metastatic disease have a statistical median survival of 8 to 12 months (Ref. 10).

While there is no known definite cause of STS, several associated or predisposing factors have been identified, (Ref. 3, Ref.11) and include:

- External radiation (STS may develop within 3 to 15 years following radiation for lymphoma, cervical, testicular or breast cancers)
- Genetic alterations such as deletions or mutations in the tumor suppressor gene p53 and the

retinoblastoma gene (Rb)
- The genetic disorder neurofibromatosis type 1, which carries a 10% lifetime risk for STS originating in the peripheral nerve sheath

**Chromosomal Alterations**
In the last two decades, the finding of specific acquired chromosomal alterations in sarcomas has helped in a proportion of cases to understand the underlying genetic basis of these tumors. These studies have allowed researchers to classify sarcomas into two main groups: (1) sarcomas with specific genetic alterations on a background of relatively few other chromosomal changes, and (2) sarcomas with no specific genetic alterations on a complex background of numerous chromosomal changes. One third of sarcomas falls in the first group and is characterized by specific recurrent genetic changes known as chromosomal translocations. As the molecular basis of these chromosomal translocation events are identified for each sarcoma, important new information has been provided that is changing how these sarcomas are diagnosed and how the prognosis of these sarcoma patients is being determined. Finally, this information will hopefully be useful in changing how these cancers are treated in the future. (Excerpted from Chromosomal Translocations in Sarcomas: New Perspectives).

The presence of STS may not always be readily apparent as these tumors commonly present as asymptomatic lesions and may be so deep as to go unnoticed, possibly resulting in delayed diagnosis (an example is in the abdomen, where they may grow to large sizes before causing symptoms and being diagnosed). STS typically presents as a solid mass, most often occurring in the extremities (43%), and next within the abdomen (34%) (Figure 1).

**Figure 1: Distribution of Soft-tissue Sarcoma by Body Area (Ref. 3)**

Distribution by body site of STS in 5312 patients aged 16 years or older admitted to Memorial Sloan-Kettering Cancer Center between July 1, 1982 and December 31, 2002.

The size of a STS tumor at diagnosis varies by body site. Tumors within the abdomen and thigh usually grow to be quite large because they often go unnoticed since there are no bony structures that can potentially limit tumor growth. However, tumors near bone, such as the head and neck...
and distal limbs, are typically much smaller, simply because they are usually noticed earlier. Patients whose tumors grow into surrounding tissues may also present earlier, due to the onset of paresthesias, edema, or other symptoms of increased pressure. The most common site for distant metastases is the lung (Ref. 3, Ref. 12).

The stage distribution based on historic data shows that 53% of soft tissue cancers are diagnosed while the cancer is still confined to the primary site (localized stage); 25% are diagnosed after the cancer has moved beyond the primary site (regional stage); 15% are diagnosed after the cancer has already metastasized (distant stage) and for the remaining 7% the staging information was unknown. The corresponding 5-year relative survival rates were: 84.7% for localized; 60.6% for regional; 16.0% for distant; and 54.2% for unstaged (Ref. 7). Patients diagnosed with metastatic disease may die from the disease with a median survival of about 8 to 12 months (Ref. 10). A recent retrospective study further demonstrated that soft tissue sarcoma patients presenting with metastatic disease have a low survival rate, but complete eradication of tumor correlated with longer survival. Longer-term studies especially those tracking the outcome of complete responders and those completely resected will help determine the efficacy of chemotherapy (Ref 44).

Surgery is the primary treatment of localized disease. In addition to surgery, patients presenting with disease confined to an extremity may also be treated with adjuvant (therapy following surgery) radiation therapy (RT) when indicated. RT impacts local recurrence. Approximately one-third of these patients eventually develop distant metastatic disease (Ref. 6, Ref. 14).

The most common metastatic route for extremity soft-tissue sarcomas is via the venous system to the lungs. Metastases to other sites, such as the brain, liver and soft tissue distant from the primary tumor, occur but are rare. More than three-quarters of patients who develop distant metastases present with disease confined to the lung. Extrapulmonary metastases usually appear after lung metastasis and represent disseminated disease (Ref. 6, Ref. 10, Ref. 14). Where soft-tissue sarcomas usually recur as lung metastasis or local recurrence, exceptions such as angiosarcoma, epithelioid sarcoma, rhabdomyosarcoma and synovial sarcoma have an atypical tendency toward regional lymph node metastasis. Liposarcomas have shown an increased incidence of initial extrapulmonary metastatic sites in some studies (Ref. 6). In cases of late stage (metastatic) STS approximately 40% to 50% of these patients cannot be cured. Surgical resection of pulmonary metastases may render selected patients free of disease (Ref. 10).

Although a patient’s localized disease may be controlled through surgery and radiotherapy, a substantial percentage of patients’ STS will eventually recur at distant sites. The use of adjuvant chemotherapy to treat adults with localized, resectable STS remains controversial. An update to a 1997 meta-analysis of randomized controlled trials (RCTs) to reassess the efficacy of doxorubicin-based chemotherapy with respect to recurrence and survival confirms the marginal efficacy of chemotherapy in localized resectable STS with respect to local recurrence, distant recurrence, overall recurrence, and overall survival (Ref. 16, Ref. 17):

This updated meta-analysis confirms the marginal efficacy of chemotherapy in localized resectable soft-tissue sarcoma with respect to local recurrence, distant recurrence, overall recurrence, and overall survival. These benefits are further improved with the addition of ifosfamide to doxorubicin-based regimens, but must be weighed against associated toxicities.
These benefits are further improved with the addition of IFOS to doxorubicin-based regimens, but must be weighed against associated toxicities (Ref. 3). For patients presenting with metastatic disease or locally advanced disease inaccessible to adequate local treatment, chemotherapy should be considered as curative treatment initially, but in the absence of meaningful initial PFS it often becomes palliative therapy.

### RR and PFS

Response rate (RR) is the portion of patients with a tumor size reduction of a predefined sum for a minimum time period. Response duration is measured from the time of initial response until documented tumor progression. The FDA has generally defined RR as the sum of partial responses (PRs) and complete responses (CRs). When defined in this manner, RR is a direct radiologic measure of the drug’s effect in reducing tumor size. As the correlation of radiologic measurement and assessment with tumor biology and treatment is better understood, effect on reduction on tumor size in many instances does not necessarily have a direct correlation to overall survival. It is important to note that stable disease is currently not included in the RR.

What has emerged in sarcoma (and several other cancers e.g.: primary HCC, lung cancer, pancreas cancer etc.) is that stable disease over time (PFS) is a good correlate or surrogate for survival. There are several caveats to this. One needs to make sure PFS is being correctly measured and assessed, and then compared to standard statistical barometers of activity. The EORTC data would strongly support that a progression-free rate of > 40% is consistent with an active drug (for both highly targeted and non-targeted drugs; i.e. this measurement is mechanism-agnostic). Stable disease is optimally evaluated in randomized trials examining progression-free survival (PFS) between two arms, one of which is a control, the other the experimental.

The progression-free survival duration is defined as the time from randomization to objective tumor progression or death. PFS is a preferred regulatory endpoint because it includes death and may correlate better with overall survival, the gold standard at FDA. FDA has accepted PFS as a surrogate for survival in sarcoma. The ongoing randomized Phase II (PICASSO) trial design has had the input of multiple sarcoma experts from both the US and EU, and conforms to what FDA would require for front-line, 2nd-line new drug approval (doxorubicin is the only FDA-approved drug; ifosfamide is not FDA-approved in sarcoma).

Chemotherapy is standard for metastatic disease that cannot be surgically resected (Ref. 14) but is consequently often only palliative (Ref. 18). A good correlate is the impact of anatomic location on the ability to apply local treatment. For example the use of adjuvant radiation therapy to reduce local recurrence in extremity STS has demonstrated improved local control over surgery alone. Death from extremity tumors, although associated with local recurrence, is almost always a result of metastatic disease (Ref 37). Most patients are offered standard therapy with doxorubicin and/or IFOS which may produce a response rate between 15% and 30% (Ref. 17). The response rate per se does not predict survival; rather the absence of progression (progression free survival or PFS) impacts survival (Ref. 19). These treatments are, however, associated with
significant toxicities, including life-threatening doxorubicin-associated cardiotoxicity (Ref. 20). Development of resistance is also a frequent complication (Ref. 21).

The toxicities observed with IFOS are those commonly seen with other antineoplastic agents such as neutropenia (low white cell count), thrombocytopenia (low platelet count), nausea, vomiting, alopecia (loss of hair), and hypersensitivity reactions. IFOS also has more specific toxicities, including hemorrhagic cystitis, neurotoxicity (encephalopathy), and nephrotoxicity (Ref. 22). Newer agents have recently been approved, but have been associated with some debilitating undesirable effects such as nausea, fatigue, vomiting, anorexia, neutropenia, thrombocytopenia, anemia (low red blood cell count), skin rash/eczema and decreased liver function (Ref. 23, Ref. 24). Dacarbazine (DTIC) is also used but has a low response rate in patients with metastatic STS (Ref. 25).

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**Yondelis, GLEEVEC and the Management of Risk**

Two examples of newly developed agents used to treat STS are Yondelis (trabectedin) and Gleevec (imatinib). While Yondelis and Gleevec are relatively well-tolerated agents, the data from the FDA label (Gleevec), and EMEA label (Yondelis) detail a number of severe adverse events. These data are borne out in subsequent multi-group and non-sarcoma trials. It is important to note that even newer drugs, including "targeted" drugs, always carry a level of risk, and often some level of significant risk associated with their use. As clinicians learn to use these new drugs, they also learn how manage and mitigate that risk.

In the case of Yondelis, (which is not approved in the United States for the treatment of sarcoma), the summary of the safety profile provided as part of the EMEA review states:

"Significant hematological and hepatic toxicity has been observed, although toxicity was usually reversible and cumulative toxicity has not been observed. The proposed posology clearly causes a higher incidence of hematologic and hepatic toxicity than the other treatment regimen. The most common Grade 3-4 AEs in the q3wk 24-h dose group were increased ALT (12%), neutropenia (12%), increased AST (8%), and dyspnea, fatigue, nausea, and vomiting (7% each). The most frequent SAEs were dyspnea, vomiting, nausea, pyrexia, and abdominal pain. Liver toxicity usually presenting as transaminitis and neutropenia are considered to be the two dose limiting toxicities. Mortality associated with hepatic injury has been rare (<1%).

"The development of rhabdomyolysis (associated with death in 5 patients (0.5%), in the integrated safety database) is of concern and the adherence to the treatment criteria in part 4.2 of the Summary of Product Characteristics (SPC) is essential. The results regarding dose reductions, cycle delays and treatment discontinuations confirm that the proposed posology is close to the threshold where a majority of patients would experience dose limiting toxicity. Altogether available safety data showed that while being manageable, the toxicity of trabectedin is undoubtedly significant."

Concerning Gleevec, the Warnings section of the FDA approved Package Insert states:
Warnings and precautions

Edema (swelling) and severe fluid retention have occurred. Your doctor will weigh you regularly and manage unexpected weight gain by drug interruption and diuretics. Cytopenias (reduction or lack of certain cell elements in blood circulation), such as anemia, have occurred. Your doctor will perform complete blood counts weekly for the first month, every other week for the second month, and periodically thereafter. In most cases, your doctor will reduce or interrupt your therapy with GLEEVEC; in rare cases, your doctor may discontinue treatment. Severe congestive heart failure and left ventricle dysfunction have been reported, particularly in patients with other health issues and risk factors. Patients with heart disease or risk factors will be monitored and treated for the condition. Hepatotoxicity (severe liver problems) may occur. Your doctor will check your liver function before beginning treatment and continue to monitor liver function as needed. Bleeding may occur. Severe gastrointestinal (GI) bleeding has been reported in patients with newly diagnosed Ph+ CML and KIT+ GIST. GI tumor sites may be the cause of this bleeding in KIT+ GIST. GI perforation (small holes or tears in the walls of the stomach or intestine), in some cases fatal, has been reported. In patients with certain conditions associated with high eosinophil levels (eg, HES, MDS/MPD and ASM), beginning GLEEVEC has been associated with cardiogenic shock/ left ventricle dysfunction. Skin reactions, such as fluid-filled blisters, have been reported with the use of GLEEVEC. Clinical cases of hypothyroidism have been reported in patients taking levothyroxine replacement during treatment with GLEEVEC. Your doctor should closely monitor your TSH levels. Long-term use may result in potential liver, kidney, and/or heart toxicities; immune system suppression may also result from long-term use.

Other agents that have been evaluated but not yet universally used, accepted or approved for use against sarcomas include the combination of gemcitabine and docetaxel (Ref. 26). In bone sarcomas, high-dose methotrexate and cisplatin are routinely utilized, but are inactive against STS. Imatinib, sunitinib, and other kinase-specific agents that are active in GIST are inactive against nearly all other sarcomas examined to date, save for the activity of some vascular endothelial growth factor (VEGF) inhibitors against angiosarcoma and hemangiopericytoma/solitary fibrous tumor.

Thus, for most patients presenting with advanced STS, the disease is incurable, with poor prognosis and short survival times. Those for whom current chemotherapy is indicated may experience debilitating side effects and reduced quality of life. In other words this is a difficult problem for the majority of these patients, and a less toxic, hopefully more effective treatment would be hugely beneficial.

Introduction to Palifosfamide

Palifosfamide (Zymafos™ or ZIO-201) references a novel composition (tris formulation) that is the functional active metabolite of ifosfamide (IFOS), a bi-functional DNA alkylator being investigated as a potential therapy for the treatment of soft tissue sarcoma (STS). These types of molecules play a major role in the treatment of unresectable sarcoma. While chemotherapy agents can be extremely toxic drugs capable of causing death or serious injury to patients if prepared, handled, or administered improperly (Ref 31), most of these drugs tend to work, and often work well, in patients with cancer. We believe chemotherapy has improved over the years.
as we learn more about the molecular biology of cancer and how to administer chemotherapy to separate clinical benefit from toxicity and positively impact on quality of life. In that sense, there is an expectation that drugs like palifosfamide can be next generation chemotherapies.

Most chemotherapeutic drugs work by impacting DNA synthesis, a process that is important to cancer cell growth and division (Ref. 32). Many of these agents may be much more effective at specific times during the cell division process. These drugs are used alone (single agent therapy) or frequently in combination with other anticancer therapies to take advantage of their different modes of attack on cancer cells (Ref 33).

Palifosfamide references a stabilized form of the active metabolite (Isophosphoramide mustard or IPM) of ifosfamide (IFOS), a member of the oxazaphosphorine family of alkylating drugs.

**What is an Alkylating Agent and how does it work?**

In order to better understand how palifosfamide works, a brief review of the normal cell cycle will help (Ref. 1). The cell cycle is the sequence of steps a cell goes through in order to divide into more cells. A cell accomplishes this task by generating a copy of its genetic material (DNA or Deoxyribonucleic Acid) and dividing into two cells. A chemotherapeutic agent may work in only one phase of the cycle (called cell-cycle specific) or be active in all phases (cell-cycle nonspecific). Most chemotherapy agents kill cancer cells by affecting DNA synthesis or function, a process that occurs throughout the cell cycle. Each drug varies in the way this occurs within the cell cycle.

A cell’s genetic material is composed of very long strings of building blocks (nucleotides). Two strands of nucleotides join together to form the double helix that represents the genetic material (Figure 2). All of the instructions for the production of many of the substances that cells and organs need, such as proteins and enzymes, are encoded in the DNA double helix. Changes to the nucleotide sequence of the DNA are called mutations. Many chemotherapy agents, such as an alkylating agent like palifosfamide, work by altering the structure of DNA. These changes disable the cells and prevent them from dividing, growing and producing the substances required by our bodies.

![Figure 2: DNA Double Helix with correctly joined nucleotide base pairs (adapted from Cancerquest)](http://sarcomahelp.org/learning_center/palifosfamide.html)
An alkylating agent is a drug that is able to introduce impairment to DNA (forming alkyl groups). They work by binding to the DNA and interfering with various processes, such as DNA replication, transcription, and base pairing. In addition, alkylation of DNA leads to DNA strand breaks and DNA strand cross-linking that prevents DNA replication. Simply, DNA alkylators hinder tumor cell growth by impacting the DNA of the tumor cells. If the alterations are not repaired, cellular activity is stopped and the cancer cell dies.

Alkylating agents represent one of the first classes of anticancer drugs to be developed and remain among the most effective and widely used of all cancer drugs in many types of cancer (Ref 40). This class of anticancer drugs is used in many types of cancer, including both solid tumors and leukemias. Some of the more common alkylating agents go by names that include: cyclophosphamide, ifosfamide, melphalan, chlorambucil, BCNU, CCNU, dacarbazine, procarbazine, busulfan, and thiotepa (Ref. 1).

These alkylating molecules work by three different mechanisms, all of which achieve the same end result - disruption of DNA function and cell death (Ref. 2). In the first mechanism an alkylating agent attaches alkyl groups (small carbon compounds) to DNA bases damaging the DNA. This alteration results in the DNA being fragmented preventing DNA synthesis and RNA transcription.

The second mechanism of action of alkylating agents is the induction of mispairing of the nucleotides leading to altered DNA. In a normal DNA double helix, A always pairs with (is across from) T and G always pairs with C. When an alkylating agent is introduced, alkylated G bases may erroneously pair with Ts. If this defect is not corrected by the cell it may lead to a permanent mutation and cell death.

The third mechanism used by alkylating agents to cause DNA damage is the formation of cross-links, or bonds, between atoms in the DNA. In this process, two bases are linked together by an alkylating agent that has two DNA binding sites. Links can be formed within a single molecule of DNA (Figure 3) or a cross-link may connect two different DNA molecules. Cross-linking prevents DNA from being separated for synthesis or transcription. This is how palifosfamide works.
IFOS is a prodrug, meaning that it has to be metabolized in order to be an active chemotherapeutic (Figure 4). Significant toxicities are associated with the inactive metabolites of IFOS, particularly acrolein and chloroacetaldehyde (CAA)(Ref 22). Since IPM is the clinically active metabolite of IFOS, no such metabolites are produced, and the molecule is consequently much less toxic.

The active drug substance of palifosfamide is isophosphoramide mustard (IPM). The chemical name of palifosfamide is N,N'-bis(2-chloroethyl) phosphorodiamidic acid. The chemical structure of palifosfamide is shown in Figure 5.

Molecular formula: C₄H₁₁N₂Cl₂O₂P
Molecular weight: 221.02

Palifosfamide is formulated by combining the tris (hydroxymethyl) amino methane (tris) salt (palifosfamide-tris active ingredient) and a number of excipients to create the injectable (and capsule) drug product. The drug product is supplied as a lyophilized powder for reconstitution with saline prior to administration.

**Oxazaphosphorine resistance and possible approaches to its circumvention**

The oxazaphosphorines cyclophosphamide, ifosfamide and trofosfamide remain a clinically useful class of anticancer drugs with substantial antitumor activity against a variety of solid tumors and hematological malignancies. A major limitation to their use is tumor resistance, which is due to multiple mechanisms that include increased DNA repair, increased cellular thiol levels, glutathione S-transferase and aldehyde dehydrogenase activities, and altered cell-death response to DNA damage. Oxazaphosphorine resistance, together with dose-limiting toxicity (mainly neutropenia and neurotoxicity), significantly hinders chemotherapy in patients. Four major
Palifosfamide PreClinical Development

The tromethamine (tris) salt of palifosfamide was developed as an alternative to the original lysine salt formulation in order to improve the storage and reconstitution stability, enhance the solubility characteristics, and increase the concentration of IPM active drug substance per vial. It was also done to mitigate the renal toxicity thought to be seen from lysine itself. Palifosfamide-lysine exists as a 1:2 ratio (approximately) of palifosfamide to lysine while the tris salt form is a 1:1 ratio of palifosfamide to tris resulting in a more stable compound. Whereas the majority of the preclinical work was conducted with the palifosfamide-lysine formulation, the basis for introducing the palifosfamide-tris formulation in to the clinic was that both formulations contained the same active pharmaceutical ingredient (API) and produced similar impurity profiles. Hence it is appropriate to rely on the earlier preclinical work performed with the palifosfamide-lysine salt formulation and the additional preclinical work conducted with the tris salt in support of the use and potential market registration of the palifosfamide-tris formulation.

Preclinical development of palifosfamide has included in vitro and in vivo studies using established cell lines and xenograft models to demonstrate activity against various sarcomas, breast cancers, other solid tumors and leukemias, including several that are resistant to IFOS (Ref. 9, Ref. 27, Ref. 28). Phosphoramide mustard (PM), isophosphoramide mustard (IPM/palifosfamide), and the lysine and tris salt forms of palifosfamide have also been compared in multiple preclinical models.

Palifosfamide-lysine was investigated in vitro for effects on the viability and proliferation of rhabdomyosarcoma (RD and RH30), Ewing’s sarcoma (SKPNDW and SKES1), osteosarcoma (Saos-2, OS222, OS29, and OS230), and synovial sarcoma (HSSYII and SYOI) human cell lines. After a 24-hour pre-treatment period, sarcoma cell lines were incubated with increasing concentrations of palifosfamide-lysine, either as a single-day treatment or as 3 consecutive days of treatment with fresh drug added each day. The treated cells were incubated for an additional 72 hours (96 total hours for 1×, and 144 total hours for 3× treatment regimens) and cell viability was...
determined by colorimetric measurement of the conversion of 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide to formazine (MTT assay). The MTT assay measures mitochondrial function and provides an estimate of cell viability after exposure to IPM. The IC50, defined as the concentration at which viability of IPM-treated cells was 50% of the viability of control cells, was determined from the dose-response curves of cell viability. Palifosfamide-lysine was found to have a cytotoxic/cytostatic effect against all of the sarcoma lines selected for investigation as shown in Table 2 below:

Table 2. IC50 Values for Dose Response of Palifosfamide-Lysine Cytotoxic/Cytostatic Activity against Human Sarcoma Cell Lines in vitro

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Histology</th>
<th>IC50 of Palifosfamide-lysine (μg/mL)</th>
<th>IC50 of IPM dose equivalent (μg/mL)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Daily×3</td>
<td>Daily×1</td>
<td>Daily×3</td>
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<tr>
<td>SYO-1</td>
<td>Synovial sarcoma</td>
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<td>Osteosarcoma</td>
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<td>0.5</td>
</tr>
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<td>Ewing’s sarcoma</td>
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<td>1.01</td>
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<td>Ewing’s sarcoma</td>
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<td>Synovial sarcoma</td>
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<td>0.45</td>
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<td>Alveolar rhabdomyosarcoma</td>
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<td>0.86</td>
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<td>RD</td>
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<tr>
<td>OS222</td>
<td>Osteosarcoma</td>
<td>1.21</td>
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</tbody>
</table>

Palifosfamide-lysine-lysine salt formulation of palifosfamide

1 IC50 = concentration at which the optical density of the treated cells is 50% of that of control cells in the MTT colorimetric assay

2 Palifosfamide (IPM) forms a salt with lysine in a 2:1 molar ratio of lysine to IPM. Thus, each 100 mg IPM-lysine salt is equivalent to 33 mg IPM free acid.

The tris salt formulation of palifosfamide (palifosfamide-tris) is more soluble and more stable in solution than the lysine salt formulation (palifosfamide-lysine) which has led to the evaluation of palifosfamide-tris for development as an oral and injectable salt of palifosfamide. The antitumor effects of palifosfamide-tris, palifosfamide-lysine and IPM were compared in the MX-1 breast carcinoma xenograft model. Two studies were performed in which the PO and IP routes of administration were investigated (Ref 35). Doses of the compounds were selected so as to allow for comparison of antitumor effects at IPM-equivalent doses for each salt of IPM. MX-1 tumor fragments were implanted subcutaneously (SC) in the mammary fat pads of athymic nude mice.

In the first study (ZP-14), IP-administered palifosfamide-lysine was toxic at the 36 mg/kg/day dose level. IP-administered palifosfamide-tris and IPM at the 36 and 24 mg/kg/day IP dose levels did not appear to cause toxicity.

In the second study (ZP-17), IP-administered IPM-tris was toxic at the highest dose (81 mg/kg/day). IP administration of palifosfamide-tris at the 24, 36, and 54 mg/kg/day dose levels and administration of palifosfamide-lysine and IPM at the 24 and 36 mg/kg/day levels did not appear to cause toxicity.

From these two studies performed in the MX-1 breast carcinoma xenograft, it can be concluded
that palifosfamide-tris elicits significant antitumor activity when administered by either the PO or IP routes with equivalent activity. IPM-tris administered IP or PO at nontoxic doses exhibited significant antitumor activity in both studies.

The antitumor activity of palifosfamide-tris in combination with doxorubicin was investigated in the MX-1 breast carcinoma xenograft model (Ref 36). Fragments of in vivo-passaged MX-1 tumors were implanted SC in the mammary fat pads of athymic nude mice. Palifosfamide-tris doses of 54, 24, or 12 mg/kg/day were administered IP for 5 days. Doxorubicin doses of 8, 5.3, or 3.5 mg/kg/day were administered IV on Days 1, 4, and 8. In the palifosfamide-tris/doxorubicin combination treatments, the agents were administered by same routes and schedules and at the same respective dose levels. Antitumor effects of the palifosfamide-tris and doxorubicin treatments were evaluated in comparison to vehicle treatment by measuring tumor growth and animal survival. Toxicity was assessed by monitoring overall body weight, appearance, and deaths unrelated to tumor burden.

A cooperative and synergistic interaction was observed between doses of 12 or 24 mg/kg palifosfamide-tris in combination with the MTD (8 mg/kg) of doxorubicin in the MX-1 xenograft. In two separate studies, combination treatment administered to mice bearing established orthotropic MX-1 xenografts inhibited tumor growth to a significantly greater extent than treatment with either agent alone. The greater antitumor activity of the palifosfamide-tris/doxorubicin combination resulted in significantly increased survival (P<0.001 [log-rank Mantel-Cox test]).

These results suggested that the palifosfamide-tris/doxorubicin combination can exhibit antitumor activity exceeding that of either drug administered as a single agent when the agents are combined at doses causing only low toxicity in mice. These data strongly supported an exploration of the combination regimen in the clinical setting.

Overcoming Limitations

In early Phase I studies with palifosfamide-lysine (ZIO-201), an original stabilized form of isophosphoramide mustard (IPM), while very safe caused a reversible Fanconi Syndrome (also termed proximal renal tubular acidosis or urinary electrolyte wasting) at higher doses (very similar to that seen with IFOS). Fanconi Syndrome is the impairment of proximal renal tubular function that is characterized by excessive urinary loss of glucose, amino acids, phosphorus, calcium, uric acid, bicarbonate, potassium, sodium, magnesium, and low molecular weight proteins.

ZIOPHARM reduced the dose of palifosfamide-lysine administered in all of its active clinical trials and the number of reported cases of Fanconi Syndrome substantially decreased. However, clinical laboratory results collected as part of the ongoing ZIOPHARM monitoring program suggested that the incidence of acute renal failure (while low) was increasing in conjunction with patients tolerating longer-term administration of palifosfamide-lysine.

ZIOPHARM developed a new formulation of palifosfamide (palifosfamide-tris) to overcome
several limitations associated with the original palifosfamide-lysine formulation. The limitations associated with palifosfamide-lysine include the need for -70°C conditions when storing long-term, short post-reconstitution stability, and a 2:1 ratio of salt to active pharmaceutical ingredient (API). Palifosfamide-tris has the capability of being stored at -20°C for at least 6 months, has twice the reconstitution stability compared to palifosfamide-lysine, and has a 1:1 salt-to-API ratio. ZIOPHARM continued to work closely with nephrology experts who believed that lysine, in of itself a nephrotoxin, especially when combined with palifosfamide, exacerbated the renal toxicity. Thus the absence of lysine would be predicted to abrogate nephrotoxicity. In addition, as ZIOPHARM gained more experience with palifosfamide in its lysine form, dosing schedule and dose adjustments were made and the observed renal toxicities changed accordingly (i.e significantly reduced, even when administering effective high-dose palifosfamide). Thus, with the change to the tris-salt form we have equivalency, and have observed a significant decrease in the number of reported renal toxicity and have experienced no Fanconi Syndrome. Indeed, in the current randomized trial it is unclear as to whether there is any difference in toxicity between the combination arm (pali + dox) and the control arm (dox alone).

Palifosfamide Clinical Development

Even though a number of agents have been evaluated as single-agent treatments or in combination with other available therapies for advanced sarcoma treatment, response rates remain low: about 5% - 27 % for doxorubicin, approximately 5% - 25% for IFOS and 5% - 20% for dacarbazine (Ref. 18). However, the response rate in sarcoma has no bearing on the ultimate survival of patients. In other words, it is not always a good surrogate for activity. In contrast, “stable disease” for a reasonable duration – “progression free survival” is a good surrogate for survival. The treatment of metastatic sarcoma is difficult, and the vast majority of patients progress and die from their disease, with median survival in patients with advanced or metastatic STS reported at around 1 year or less (Ref. 10, Ref. 18).

It is clear that sarcoma patients have critical unmet medical needs that have gone largely unfulfilled: few options exist for those who fail front-line therapy and existing second-line therapies offer only minimal efficacy. Existing treatments and combinations are often quite toxic, and frequently very difficult to tolerate.

As mentioned earlier, a disadvantage of IFOS is that it is a pro-drug and must be metabolized in order to be active. Its clinical utility is limited by toxicities associated with metabolites unrelated to DNA-alkylation as well as by development of resistance due to decreased pro-drug activation. Palifosfamide, formulated as a tris/mannitol salt conceptually lacks toxicities that are associated with IFOS treatment, with no brain toxicity, no renal problems and no hemorrhagic cystitis. Unlike IFOS, palifosfamide does not require co-administration of mesna or hydration therapies, resulting in reduced overall treatment time and potentially much less expense associated with in hospital treatment and much less post-treatment expense. It is also much easier to administer, and can usually be safely done on an out-patient basis.

Palifosfamide as a Single Agent Therapy
ZIOPHARM is actively pursuing palifosfamide as a therapy in STS. Several clinical studies have been initiated in a variety of cancer types. A single arm, non-randomized, open label Phase I study in advanced cancers (Study IPM1001) using the original lysine salt formulation has been completed. The study design called for multiple 3-week cycles consisting of either daily administration of palifosfamide for 3 days and repeated every 21 days, or a single administration of palifosfamide given every 21 days. Patients were treated up to 6 cycles, or until dose-limiting toxicity (DLT) or disease progression occurred. Dose levels of palifosfamide were initially increased in successive cohorts of single patients for the first 8 cohorts (dose levels of 30 mg/m^2, 42, 59, 83, 116, 162, 227, and 318 mg/m^2) and then 3 patients per dose level for the remaining cohorts. At each dose level, the first 3 patients were treated and evaluated for toxicity over a 3-week post-dosing observation period. The most frequently reported events include nausea, fatigue, and anorexia (Ref. 9). There have been no significant hepatic or cardiac toxicities reported.

A single arm, non-randomized, open label, two-stage Phase I/II Study in advanced sarcomas (Study IPM2001), introducing the tris salt formulation, has completed and data retrieval is ongoing. The study design called for multiple 3-week cycles that consisted of daily doses on 3 consecutive days repeated every 21 days. Patients were treated for up to 6 cycles or until dose-limiting toxicity (DLT) or disease progression occurred. The Phase I starting dose was 590 mg/m^2. Four patients were enrolled at this dose level. This starting dose was found to be too high, resulting in renal toxicity. Per protocol, the dose was reduced to 413 mg/m^2, which proved to be the maximum tolerated dose. The Phase II portion of the study was conducted using the 413 mg/m^2 dose level. This study also incorporated a single IV dose of palifosfamide-tris (the new formulation, replacing lysine) for bioequivalence evaluation.

Preliminary safety results from the Phase I portion (Table 3) (Ref. 28, Ref. 29) along with preliminary efficacy data for the Phase II portion of Study IPM2001 (Table 4) have been reported (Ref 30). The most frequently reported adverse events from Phase I include nausea (44%), hypokalemia (44%), fatigue (32%), anemia (34%); hypophosphatemia (32%), increased blood alkaline phosphatase (32%), increased blood creatinine (24%); microhematuria (24%); vomiting (22%); and hypocalcemia or increased blood lactate dehydrogenase (20% each). There were no significant hepatic or cardiac toxicities reported. With the new tris/ mannitol formulation there has been little renal toxicity reported and the reported hematuria is by dipstick; most likely related to common urological changes associated with chemotherapy in general, such as urinary tract infection, as opposed to hemorrhagic cystitis which is seen with IFOS.
In Phase II, one partial response lasting 35 weeks was reported in a patient diagnosed with liposarcoma (Figure 6). In addition, in heavily pretreated patients (second line and above with a median of 5 prior chemotherapies), the progression-free survival (PFS) at 3 months was 43% for all patients, for patients with STS the rate was 45%, and for those patients that were IFOS-naïve, the rate was 55%, warranting further evaluation and suggesting drug activity in sarcoma. These data are strongly consistent with single agent activity of palifosfamide in soft tissue sarcoma. (Ref 30)

Table 3: Most Common (>10%) Phase I Adverse Events
(Safety Population; N=41)

<table>
<thead>
<tr>
<th>Frequency (%)</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypokalemia</td>
<td>18 (44)</td>
</tr>
<tr>
<td>Nausea</td>
<td>18 (44)</td>
</tr>
<tr>
<td>Anemia</td>
<td>14 (34)</td>
</tr>
<tr>
<td>Alkaline phosphatase increased</td>
<td>13 (32)</td>
</tr>
<tr>
<td>Fatigue</td>
<td>13 (32)</td>
</tr>
<tr>
<td>Hypophosphatemia</td>
<td>13 (32)</td>
</tr>
<tr>
<td>Elevated creatinine</td>
<td>10 (24)</td>
</tr>
<tr>
<td>Microhematuria</td>
<td>10 (24)</td>
</tr>
<tr>
<td>Vomiting</td>
<td>9 (22)</td>
</tr>
<tr>
<td>Lactate dehydrogenase increased</td>
<td>8 (20)</td>
</tr>
<tr>
<td>Hypercalcemia</td>
<td>8 (20)</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>8 (20)</td>
</tr>
<tr>
<td>CO₂ decreased</td>
<td>6 (15)</td>
</tr>
<tr>
<td>Constipation</td>
<td>6 (15)</td>
</tr>
<tr>
<td>Urinary tract infection</td>
<td>6 (15)</td>
</tr>
<tr>
<td>Dyspnea</td>
<td>5 (12)</td>
</tr>
</tbody>
</table>

Table 4: Preliminary Phase II Single Agent Efficacy Results
Progression-free Survival (PFS)

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>PFS at 3 Months (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>41</td>
<td>43</td>
</tr>
<tr>
<td>STS</td>
<td>33</td>
<td>45</td>
</tr>
<tr>
<td>IFOS-naïve</td>
<td>11</td>
<td>55</td>
</tr>
</tbody>
</table>

Note: Actuarial PFS at 6 months for STS patients with palifosfamide: 23%.

In Phase II, one partial response lasting 35 weeks was reported in a patient diagnosed with liposarcoma (Figure 6). In addition, in heavily pretreated patients (second line and above with a median of 5 prior chemotherapies), the progression-free survival (PFS) at 3 months was 43% for all patients, for patients with STS the rate was 45%, and for those patients that were IFOS-naïve, the rate was 55%, warranting further evaluation and suggesting drug activity in sarcoma. These data are strongly consistent with single agent activity of palifosfamide in soft tissue sarcoma. (Ref 30)
In studying a potential anticancer agent, a number of criteria may be used to assess early antitumor activity. Because tumor response rates may be included as endpoints in cancer clinical trials, validated response criteria can be key to the success of a trial and ultimately to the approval of an anticancer agent (Ref 38). The majority of cancer clinical trials are conducted at multiple institutions, so both the response criteria and the tools used for evaluating response must be consistent across multiple centers. Traditional response criteria are based on changes in tumor size. However, such anatomic criteria can be difficult to apply across multiple institutions when measuring certain tumors, in particular soft tissue sarcomas. In addition to being potentially difficult to measure, soft tissue sarcomas may not necessarily change in size in response to therapy, at least initially. Including a decrease in tumor burden measured against a predetermined, standardized scale (Ref 38). These ‘predetermined decreases’ in tumors are most often categorized using a coding system referred to as RECIST (Response Evaluation Criteria in Solid Tumors). However, in sarcoma this scale has many limitations and the European Organization for the Research and Treatment of Cancer (EORTC) and others have suggested it is more accurate and predictive to evaluate an investigational agent’s potential effect on cure against advanced sarcoma using other criteria, in particular the progression-free rate. The EORTC criteria for evaluating second-line therapies suggests that investigational anticancer agents with a 3-month PFR of ≥39% indicates drug activity, while a PFR of ≤21% indicates inactivity (Ref 42).

**Palifosfamide in Combination with Doxorubicin (Adriamycin)**

Preclinical studies have shown marked synergy when palifosfamide was combined with doxorubicin. These synergies were seen at low and intermediate doses of both agents without added toxicities (Ref. 36).

Doxorubicin as single agent or part of a combination therapy is currently the standard of care used to treat patients with a number of malignancies including advanced sarcoma. However, its use is limited by associated toxicities and limited efficacy. Importantly, the toxicities observed...
with palifosfamide do not appear to overlap the toxicities noted with doxorubicin. The unique
Toxicity profiles of these two agents plus the positive preclinical and clinical data make this an
Appropriate chemotherapeutic regimen to evaluate.

ZIOPHARM initiated a Phase I, open-label, non-placebo-controlled, non-randomized, dose
Escalating study to define the safety profile, pharmacokinetics, pharmacodynamics, and tumor
Response profile of palifosfamide in combination with doxorubicin (See Table 5). Eligible
Patients with advanced, refractory solid tumors, for which no standard therapy exists and for
Whom treatment with doxorubicin is considered medically acceptable, were included. The
Objectives of this Phase I study were to define the safety profile of palifosfamide when
Administered in combination with doxorubicin, to evaluate the pharmacokinetics and
Pharmacodynamics of palifosfamide in combination with doxorubicin, and to determine the
Objective tumor response rate, including complete and partial responses as measured by response
evaluation criteria in solid tumors (RECIST) in patients with evaluable disease.

Table 5: Dose Escalation Schedule

<table>
<thead>
<tr>
<th>Dose Cohort</th>
<th>Doxorubicin (mg/m²)</th>
<th>Palifosfamide (mg/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>60</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>60</td>
<td>150</td>
</tr>
<tr>
<td>3</td>
<td>75</td>
<td>150</td>
</tr>
</tbody>
</table>

- Palifosfamide administered over 30 minutes on day 1,2,3 of each 21 day cycle.
- Administered without Mesna, and without hydration.
- Doxorubicin on day 1 only. 1 cycle = 21 days.

Preliminary results from 13 metastatic patients (including diverse histologies: MFH, MPNST,
RMS, endometrial stromal sarcoma, leiomyosarcoma, osteosarcoma, angiosarcoma,
neuroendocrine carcinoma, adrenal carcinoma, mesothelioma, SCLC) are available (Ref. 30). All
Had ECOG PS <2, the median age was 58, and 3 had received prior IFOS.

With 51 cycles administered at the time of this preliminary analysis, the combination has been
Well tolerated, with no dose-limiting toxicities reported. Study-related Grade 3/4 adverse events
(AEs) include neutropenia and thrombocytopenia, considered possibly related to both drugs. Of
The 149 AEs reported, 8 were possibly related to palifosfamide and 12 were possible related to
doxorubicin. Thirty-two (32) AEs were possibly related to both drugs, with 7 AEs definitely
Related to doxorubicin and none definitely related to palifosfamide. Figure 7 and Table 6
Summarize an overview of the safety profile for palifosfamide to date. Other events include
Anemia, microhematuria (infection related), nausea, and vomiting. There have been no reported
Incidence of encephalopathy, no hemorrhagic cystitis, and no renal toxicity.
Of the 13 patients included in the preliminary analysis, 12 patients have received at least 2 cycles of therapy and are evaluable for efficacy using RECIST criteria. All of the STS patients (8) are evaluable with 2 patients from this sarcoma subset reporting a Partial Response (PR – see Figures 8 and 9), and the remaining 6 patients reporting Stable Disease (SD). The mean time on study is 14 weeks.
In summary, preliminary results from this ongoing Phase I study shows that palifosfamide in combination with doxorubicin is well tolerated. There have been no significant bladder or neurotoxicity reported and no renal toxicity either as a single agent or in combination. Initial combination efficacy in STS reveals 2 out of 8 PR with current median duration of follow up of 20 weeks.

**Orphan Drug Designation—US and Europe**

Orphan Drug Designation is the granting of special status to a product to treat a rare disease or condition. In order for a developer to obtain orphan designation, an application must be submitted to the appropriate governmental organization, and the application for designation must be approved. The approval of an application for orphan designation is based upon the information submitted by the developer demonstrating that there is a real potential for the product to have an impact on the targeted disease or condition. A product that has obtained orphan designation is said to have "orphan status. Each designation request must stand on its own merit. The granting of orphan designation does not alter the standard regulatory requirements and process for obtaining marketing approval. Safety and efficacy of a product still must be established through adequate and well-controlled studies. However, the granting of orphan status can help accelerate the development and approval of products that demonstrate promise for the diagnosis and/or treatment of rare diseases or conditions The United States Food & Drug Administration (FDA) granted Orphan Drug Designation to palifosfamide in the treatment of Soft Tissue Sarcoma (STS) in May 2008. The European Medicines Agency (EMEA) granted Orphan Drug Designation to palifosfamide in the treatment of Soft Tissue Sarcoma (STS) in December 2008.

As a result of the data generated in these studies, the company initiated a Phase II randomized controlled trial comparing palifosfamide plus doxorubicin vs. doxorubicin in the front- and second-line treatment setting of STS.

Following further preclinical study and initial results from the ongoing randomized Phase II trial,
an oral form of palifosfamide is expected to enter Phase I study in solid tumors.

**Randomized Controlled Clinical Study**

Randomized controlled trial: (RCT): A study in which people are selected at random (by chance alone) to receive one of several study medications. One of these medications is referred to as the control. The control may be a standard practice, a placebo ("sugar pill"), or no intervention at all. RCTs seek to measure and compare the outcomes after the study participants receive the study medication. Because the outcomes are measured, RCTs are quantitative studies.

**PICASSO: Phase 2 Randomized Control Trial now enrolling in Soft-Tissue Sarcoma**

Based on the result of the Phase I combination study, a randomized Phase II trial comparing palifosfamide in combination with doxorubicin versus doxorubicin alone in 1st and 2nd line patients with advanced STS has been initiated. ZIOPHARM Oncology, Inc. has launched its global Phase II randomized controlled trial of palifosfamide in patients with unresectable or metastatic soft-tissue sarcoma; A Phase II multicenter, parallel group, randomized study of palifosfamide plus doxorubicin versus doxorubicin alone in patients with unresectable or metastatic Soft Tissue Sarcoma (PICASSO). The primary objective of the study is to assess the difference in progression-free survival (PFS) between patients treated with palifosfamide plus doxorubicin versus doxorubicin alone. A secondary objective is to assess the safety, tolerability and the response of the patients to both regimens treated in the study. Palifosfamide has demonstrated activity in treating patients with soft tissue sarcoma (STS) alone and in combination with doxorubicin, which suggests it may offer patients a better option than the current standard of care which is doxorubicin alone.

**Study Design**

- Multi-center randomized controlled trial with numerous US and European sites.
- Randomization into 1 of 2 arms either to receive palifosfamide plus doxorubicin or to receive doxorubicin alone.
- After 6 cycles of doxorubicin, patients will be discontinued from doxorubicin in both arms as the cumulative dose will be considered the maximum upper limit.
- Patients randomized to the palifosfamide/doxorubicin arm may continue treatment with palifosfamide beyond 6 cycles until patients are taken off the drug due to side effects or disease progression.
- Patients in the single-agent doxorubicin arm who progress at or before cycle 6 will be given the option of receiving single agent palifosfamide.

**Eligibility Criteria**

- Eligible patients have been diagnosed with advanced, histologically confirmed STS excluding alveolar soft-part sarcoma, chondrosarcoma, dermatofibrosarcoma, Ewing sarcoma, GIST, Kaposi sarcoma mixed mesodermal sarcoma, osteosarcoma, radiation induced sarcoma and...
unresectable low-grade liposarcoma.

- Prior therapy with ifosfamide is acceptable.
- Age > 18 years.
- ECOG performance status 0 or 1.

We expect to present interim data from this study at the upcoming CTOS meeting in Miami, USA in November 2009. If these data are positive, it could form the basis of a larger Phase III randomized trial to be used for potential FDA approval of palifosfamide in sarcoma.

**Conclusions**

Palifosfamide is a novel molecule being evaluated in STS. It has shown early promise in the research lab and early human testing. It is now being tested in the randomized Phase II setting in combination with doxorubicin. Numerous drug combinations have been explored for efficacy and activity in advanced STS. As combinations containing at least 1 active compound can always be anticipated to show some activity, such combinations should be explored in a randomized setting to avoid patients’ selection bias. In the randomized setting, combinations of doxorubicin have not improved PFS, overall survival, nor toxicity over single-agent doxorubicin. Therefore, a regimen that improves upon single-agent doxorubicin would address this highly unmet need. This would be of particular value if the new agent was relatively non-toxic.

Despite the fact that numerous randomized combination studies have been conducted, a meta-analyses of the doxorubicin data concluded that doxorubicin monotherapy can still be considered the standard therapy for adult patients with metastatic STS. In randomized studies where PFS is reported, single-agent doxorubicin has a short median PFS of about 3 months; thus there is an urgent need to improve upon the clinical efficacy of doxorubicin administered alone.

Palifosfamide has shown preliminary clinical evidence of activity as a single-agent in patients diagnosed with advanced sarcomas who have received at least 2 prior chemo-regimens. Preliminary results from a single-agent study conducted in patients with a broad range of histological sarcoma subtypes confirmed that palifosfamide was well tolerated. Toxicities, mainly renal, have been manageable (not currently observed with the new formulation), with no reports of central brain or bladder toxicities and without any significant bone marrow suppression or alopecia. The toxicities do not appear to overlap with those associated with doxorubicin.

**In summary:**

- Preclinical and preliminary clinical data suggest that palifosfamide will be active against a number of cancers, including sarcoma as well as a number of cancers resistant to IFOS. In some preclinical testing the activity of palifosfamide is superior to that of IFOS.
- Palifosfamide provides antitumor activity without the toxic metabolites of IFOS, in particular CAA, which is thought to induce brain, bladder and nephrotoxicity.
- In preclinical studies palifosfamide in combination with doxorubicin has shown enhanced activity at low and intermediate doses of both agents without added toxicities. Preliminary results from ongoing clinical trials support these preclinical results.
The studies outlined above, support the hypothesis that palifosfamide may be effective against soft tissue sarcoma with fewer toxicities and the potential to overcome tumor resistance compared to currently available therapies. Specifically, significant benefits are anticipated compared to current treatment.

Additional Information

- Trial information is posted at SarcomaHelp.org and ClinicalTrials.gov.
- Additional trial information can be obtained from Jill Buck at 617-259-1984.

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